40* Aztreonam lysine for inhalation (AZLI) for CF patients with *P. aeruginosa* (PA) infection

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Background/Methods: This was a Phase 3, double-blind, randomized, placebo (PL) controlled study of AZLI, a novel, inhaled formulation of aztreonam. Patients received 28 days of tobramycin solution for inhalation (TSI) followed by 28 days of 75 mg AZLI or PL (1 mL) administered BID or TID by the PARI eFlow® electronic nebulizer over ~2 minutes. Inclusion criteria included age ⩾6 years, PA in sputum or throat swab, FEV_1 ⩾25% to ⩽75% predicted, and use of ⩾3 courses of TSI in the prior year. Concomitant standard CF therapies were allowed. The primary endpoint was time to need for IV or inhaled antipseudomonal antibiotics for predefined symptoms of pulmonary exacerbation (increased cough, decreased exercise tolerance, increased sputum/chest congestion, and/or decreased appetite).
Results: 246 patients enrolled and received TSI; 211 subsequently received either AZLI (n = 135) or PL (n = 76) (mean age 26 years, mean FEV1 55% predicted). The median time to antibiotic need was at least 21 days longer for AZLI-treated patients compared to PL-treated patients ($p < 0.05$). After 28 days of treatment, FEV_1 improved by 6.3% and PA CFU density decreased by 0.7 $\log_{10}$ for AZLI-treated patients compared to PL-treated patients ($p < 0.05$). AZLI-treated patients had significant improvement in respiratory symptoms, with mean improvement of >5 compared to PL in CFQ-R respiratory domain score ($p < 0.05$). AZLI was safe and well tolerated. The adverse event profile was consistent with CF lung disease; the most common AE was cough.
Conclusion: AZLI is a promising new therapy to improve respiratory symptoms and FEV_1 in CF patients with PA.
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41* PTC124 activity in CF patients carrying stop mutations: results of a phase 2 study

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Aim: PTC124 is a molecule that promotes ribosomal read-through of nonsense mutations in mRNA. The pharmacological activity of PTC124 was determined in CF patients carrying stop mutations.
Methods: Patients received 2 cycles of oral PTC124 comprising 3 times per day (TID) dosing at 4, 4, and 8 mg/kg for 14 days and no treatment for 14 days, and then TID dosing at 10, 10, and 20 mg/kg for 14 days and no treatment for 14 days. Nasal potential difference (NPD), pulmonary function, weight and CF symptoms were assessed before and at the end of treatment in each cycle.
Results: 23 patients (median age: 25, range: 18–57 yrs; genotypes included G542X/W1282X [1], G542X/delta F508 [3], G542X/N1303K [1], W1282X/W1282X [3], W1282X/3849+10C to T [1], 3849+10C to T/delta F508 [1], and W1282X/delta F508 [13]) received both dose levels. At both doses, mean chloride conductance (average of the 2 nostrils) improved from +1.1 to −5.8 mV ($p \leqslant 0.0001$) and from −0.3 to −3.9 mV ($p = 0.032$), and normalized to >−5 mV in 56% (13/23) and 35% (8/23) of patients, respectively. At the end of low-dose treatment, mean FEV1 increased from 63.6.6 to 66.7% of normal ($p = 0.02$), FVC increased from 77.0 to 80.1% of normal ($p = 0.01$), and mean body weight increased from 57.0 to 57.7 kg ($p \leqslant 0.001$). Side effects were minimal.
Conclusions: Treatment with PTC124 in CF patients carrying stop mutations is associated with improvements in NPD, pulmonary functions, and weight. Longer term studies are in progress.

42* Validating assays on invasive airway samples as end-points for gene therapy trials.

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We are working towards a trial of CFTR gene therapy, designed to look for clinical benefit. A strong focus has been on the development of molecular, functional and clinical end-points, which we are validating in a series of observational studies. Several assays are designed for samples obtained by invasive procedures. This study was performed to assess the feasibility of obtaining and processing samples for such assays and to allow CF/non-CF comparisons.
Healthy volunteers (HV) underwent a bronchoscopy, nasal biopsy and brushing. 16/20 HV screened were included (2 CF heterozygotes, 1 pregnant, 1 withdrew). We did not consider it ethically justifiable to ask a CF subject to undergo a research bronchoscopy and therefore obtained samples (5 endobronchial biopsies & 5 bronchial brushings) from those undergoing a clinical bronchoscopy or a general anaesthetic. Bronchial samples were obtained from 15 (14 children), nasal biopsies from 6 (adults) and nasal brushings from 21 (11 adults). There were no complications of any procedure.
Of the assays attempted, some could not be performed (short circuit current measurements on biopsy samples), others were shown to need further refinement (immunohistochemical staining for CFTR protein) and others were successful (airway surface liquid height measurements, microarray & TaqMan analysis of gene expression, bacterial adherence).
The study achieved its aims of allowing us to exclude some assays from the clinical trial protocol, focus on refining others and use those successful assays to perform power calculations for detecting change after a therapeutic intervention.
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43 Lentiviral vector-mediated expression of RNA interference sequences for ENaC in cell models of human airway epithelium

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In cystic fibrosis (CF) the hyperactivity of the epithelial sodium channel (ENaC) leads to the depletion of the perciliary liquid layer and the inhibition of mucus clearance, which results in chronic infection of the airways by opportunistic pathogens. We developed a HIV-based lentiviral (LV) vector containing a siRNA cassette to efficiently knockdown the expression and activity of ENaC in human respiratory cells. A siRNA cassette (alphaA2) for the alpha subunit of human ENaC was cloned in a LV vector carrying the NGF-R (Nerve Growth Factor Receptor) marker gene. H441 cells, derived from lung adenocarcinoma, were incubated with various doses of the LV vector and the conditions that gave rise to high efficiency transduction (64–91%) were determined. Accordingly, H441 cells were transduced with the alphaA2 siRNA-LV (2000 M.O.I.); 96 hours later ENaC genes were induced with dexamethasone (DEX, 50 nM, 6 hours) and alpha, beta and gamma subunit mRNA content was determined by quantitative real time PCR. We demonstrated that alphaA2shRNA-LV transduction induced inhibition of alphaENaC mRNA down to 40–50% both in un-induced and induced conditions. Reduction in the amount of beta and gamma mRNA was also observed. Our study shows that a LV vector can down-regulate the expression of alpha, beta and gamma ENaC subunit mRNA in H441 cells. To further characterize the potential of LV-mediated down-regulation of ENaC, functional studies are under way.
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